EFFECTS OF RIBOSE SUPPLEMENTATION IN THOROUGHBRED HORSES PERFORMING A STANDARDIZED EXERCISE TEST ON THE TREADMILL

By

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EFFECTS OF RIBOSE SUPPLEMENTATION IN THOROUGHBRED HORSES PERFORMING A STANDARDIZED EXERCISE TEST ON THE TREADMILL

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Three experiments were designed to investigate the effects of ribose supplementation on blood and muscle metabolites and performance in Thoroughbred horses performing a standardized exercise test (SET). In Experiment 1, six horses were supplemented with either 0.07 g glucose/kg body weight (BW) or 0.07 g ribose/kg BW top dressed on the feed twice daily. After 2 weeks supplementation, a SET was performed. Blood was collected before, during, and after exercise; and it was analyzed for ammonia, lactic acid, glucose, ATP, pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^-. Muscle samples were collected before and after exercise and analyzed for glycogen and lactic acid. Volume of oxygen consumption (VO_2), heart rate, and performance were also recorded. After 1 week washout, the horses switched treatment groups. After another 2 weeks supplementation, a second SET was performed, and the same data were recorded. Blood ammonia was lower in the ribose treatment group at 30 minutes post-exercise ($P < 0.05$). No other differences ($P > 0.05$) were observed between treatment groups. In Experiment 2, one hour before a SET, five horses received either 250 g of ribose dissolved in 3 L of water, or 3 L of water via a nasogastric tube. After 2 weeks washout, horses switched treatment groups and a second SET was performed. The same data as in Experiment 1, except for muscle biopsies and blood ATP concentrations, were obtained. There were no differences ($P > 0.05$) between treatment groups. In Experiment 3, eight horses were supplemented with either 0.15 g glucose/kg BW or 0.15 g ribose/kg BW top dressed on the feed twice daily. After 2 weeks of supplementation, a SET was performed, and measurements for ammonia, lactic acid, glucose, VO_2, heart rate, and performance were recorded. After 10 days washout, horses switched treatment groups, and the same data were obtained. There were no differences ($P > 0.05$) between treatment groups. The results from these experiments show that ribose supplementation did not influence anaerobic exercise capacity or metabolic markers in trained horses as evaluated by these protocols.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Introduction

Numerous nutritional supplements have recently flooded the market. One such supplement is the pentose sugar, ribose. Because ribose can be assimilated into the nucleotide adenosine triphosphate (ATP), claims have been made that ribose supplementation can potentially increase anaerobic performance. Ribose is metabolized via the pentose phosphate pathway, and research indicates that ribose supplementation can increase the production of new nucleotides by increasing the *de novo* synthesis of ATP. Investigators have also reported that ribose can be used to increase the recovery of lost nucleotides through the salvage pathway.

Numerous reports indicate that decreased availability of ATP may contribute to the onset of muscular fatigue, because low ATP concentrations are associated with loss of contractile properties. Research has shown that ATP is depleted in certain equine muscle fibers after intense track or treadmill exercise. Several researchers have investigated ways to increase ATP production by using a variety of supplements, but their efforts have resulted in limited success. Whether ribose can be used to increase ATP levels and improve performance in the equine species is currently unknown. If supplemental ribose can enhance ATP synthesis, then the high energy bonds present in ATP could be used to fuel muscular work during exercise.

Performance Evaluation

Elite athletes, such as Thoroughbred horses, demonstrate great competitiveness, or will to win. Bad management can undoubtedly diminish the competitive spirit of a horse, but there is some question whether this characteristic can be enhanced. Horses possessing a strong will to win have an intrinsic desire to dominate other horses during a race; and they are able to continue to exercise when physiologic and/or psychological inputs signal a reduction in performance (Hodgson and Rose, 1994).

Elite athletic performance is not only the result of optimal function of key body systems, but also the capacity to develop resistance to signs that signal the body to reduce the exercise intensity and delay the onset of fatigue. Fatigue is exhibited by an inability of the horse to continue exercise at the intensity required. Fatigue is a complex process that appears to involve central (psychological/neurological) and peripheral (muscular) contributions. In horses, the peripheral aspects of the process have been studied in more detail than the central contributions; possibly because peripheral aspects are easier to define (Hodgson and Rose, 1994; Marlin and Nankervis, 2002).

Fitness and performance capacity are important for racehorses, although they are difficult to evaluate objectively and reliably (Rose and Christley, 1995). Various techniques, such as a SET and a track test, have been used to test the effects of supplements/drugs on the athletic capacity of horses and to evaluate performance. The SET is the most frequently cited technique in studies related to physiological changes in the exercising horse (Seeherman and Morris, 1991; Sloet van Oldruitenborgh-Oosterbann and Clayton, 1999). While not identical to exercise in a competitive race, the SET provides a method of assessing exercise capacity in an objective manner when the level of exercise is documented by accurate measurement of oxygen consumption, heart rate, and blood or plasma lactic acid concentration (Persson, 1983; Hodgson and Rose, 1994; Schuback et al., 1999).

The basic requirements of all forms of exercise testing are standardization and repeatability. These requirements are more easily met on a treadmill in a laboratory than on a track. However, there are significant differences between track and treadmill exercise tests, not only in the workload, but also in biomechanical variables in locomotion (Seeherman and Morris, 1991; Schuback et al., 1999; Sloet van Oldruitenborgh-Oosterbann and Clayton, 1999).

During track tests many environmental influences can affect performance, especially the weather and the track surface. During treadmill tests speed is easily controlled, and it is possible to use more invasive techniques during exercise. However, one of the issues in using the treadmill test is the habituation of the horse to the treadmill. The horses must adjust to the effects of differences in footing, and sometimes, the incline of the treadmill surface (Buchner et al., 1994). For a track exercise test, horses only need a short period of habituation to the measurement conditions, while a much longer period is necessary for treadmill tests. A second issue is that horses on the track usually are exercised with a rider, while they exercise unmounted on the treadmill.

Research describing differences in metabolic response to track and treadmill exercise contradict each other, possibly because of differences in habituation period, workload, and physical condition of the subjects (Scheffer and Sloet van Oldruitenborgh-Oosterbann, 1996). In equine research, the most commonly used variables to determine the workload are heart rate, plasma lactic acid, and oxygen uptake (Persson, 1983). Since oxygen uptake is very difficult to measure on a track, heart rate and plasma lactic acid are the two most common variables used in determining and comparing the workload of a horse during track and treadmill exercise.

Barrey et al. (1993) reported that horses ridden on the track had significantly higher heart rate and blood lactic acid concentration than horses exercised unmounted on a horizontal treadmill at 10 m/s. However, a 3.5% incline of the treadmill resulted in heart rates and blood lactic acid concentrations comparable to those of the track test. In another study, Sloet van Oldruitenborgh-Oosterbann and Barneveld (1995) reported the workload of horses assessed by heart rate and plasma lactic acid concentration, ridden by the same rider, both on the track and on a horizontal treadmill. The authors stated that the workload of the horses exercised on the track was significantly higher than that of the horses exercised on the treadmill. Increasing the speed of the treadmill by 10% resulted in heart rates and lactic acid concentrations comparable to those of the track test. Inclining the treadmill by 1 or 2% had a less consistent effect than increasing the speed of the treadmill (Sloet van Oldruitenborgh-Oosterbann and Barneveld, 1995).

The choice between track and treadmill tests depends primarily on the aim of the test and the variables to be measured. The main advantage of the track test is the shorter habituation period. However, treadmill tests are easier to standardize and, therefore, are more repeatable. For this reason, treadmill tests are usually preferable for most research purposes (Sloet van Oldruitenborgh-Oosterbann and Clayton, 1999).

Adenine Nucleotide Metabolism

The metabolic rate of active skeletal muscle can be several hundred times greater during muscular activity than at rest (Snow et al., 1985; Schuback and Essen-Gustavsson, 1998; Hellsten et al., 1999). One reason for the increased metabolism is to sustain the ATP production required by the working muscles. Equine studies have shown that exhaustive exercise causes muscle ATP levels to drop significantly below resting values, because ATP is used at a higher rate than the maximum capacity of the muscle to rephosphorylate adenosine diphosphate (ADP) to ATP (Essen-Gustavsson et al., 1999). For muscular contractions to continue, the body must maintain a high ATP:ADP ratio (Schuback and Essen-Gustavsson, 1998; Schuback et al., 2000).

During exercise, ATP can be generated via the adenylate kinase reaction by combining two molecules of ADP to form a molecule of ATP and a molecule of adenosine monophosphate (AMP), 2ADP → ATP + AMP, (Lehninger et al., 1992; Voet and Voet, 1995; Zubay, 1998). AMP can then be deaminated by AMP deaminase to inosine monophosphate (IMP) and ammonia (Harris et al., 1987; Graham et al., 1990; Essen-Gustavson et al., 1997; Ogino et al., 2000). During exercise, the K_m value of AMP deaminase is substantially lowered, increasing the activity of AMP deaminase and allowing for a faster rate of deamination of AMP (Sahlin et al., 1978; Shiraki et al., 1979; Bangsbo et al., 1992; Rundell et al., 1992).

Studies have also shown that the activity of AMP deaminase is enhanced by increased concentrations of ADP, AMP, and H^+, whereas high concentrations of ATP inhibit the enzyme (Wheeler and Lowenstein, 1979; Dudley and Terjung, 1985; Lowenstein, 1990). The anaerobic pathway of glycolysis is also increased by the presence of high levels of AMP (Lehninger et al., 1992; Hellsten et al., 1999). A high rate of glycolysis leads to the formation of lactic acid and H^+, which activates AMP deaminase. The accumulation of ammonia, which is almost exclusively formed by the action of AMP deaminase, indicates that ATP is being produced via the adenylate kinase reaction; and that there is a failure of the normal metabolic pathways in the production of ATP. This may contribute to fatigue, impairing the equine athlete's performance (Meyer at al., 1980; Sewell et al., 1992; Rasamen et al., 1993; Graham and MacLean, 1998; Harris et al., 1999).

Ammonia has a low molecular weight and is rapidly released from the muscle. It has been reported that an increase in ammonia concentration correlates with the loss of ATP during exercise (Harris et al., 1990, 1991). Harris et al. (1997) reported that ammonia accumulation in the blood is preferable to other catabolites (i.e., hypoxanthine, uric acid) for indicating adenine nucleotide degradation. Sewell and Harris (1992) reported a high correlation between IMP and ammonia ($r = 0.94$). The close association between ammonia and IMP suggests that adenine nucleotide degradation is the primary source of ammonia due to anaerobic exercise.

IMP can be reaminated back to AMP via the purine nucleotide cycle. However, some IMP is further catabolized to inosine, which can diffuse out of the myocyte into the extracellular space where it is no longer available as a precursor for immediate ATP replenishment. The loss of these metabolites can result in a net loss of the total adenine nucleotide pool (Bangsbo et al., 1992; Hellsten, 1994; Hellsten et al., 1999; Brault and Terjung, 2001). Therefore, loss of the adenine nucleotides from the muscle requires compensation if the muscle's ATP is to return to pre-exercise levels.

The degradation of ATP and the total adenine nucleotide pool increases during exercise, and it is most extreme during intense exercise. Repeated days of intense exercise lead to a deficit of the total adenine nucleotide pool in rested muscle (Arabadjis et al., 1993; Hellsten et al., 1993a; Hellsten et al., 1998; Hellsten et al., 1999). Hellsten et al. (1993b) reported that 1 week of high-intensity exercise significantly decreased the levels of both ATP and the total adenine nucleotide pool in skeletal muscle. Stathis et al. (1994) reported that resting ATP and total adenine nucleotide pool levels were lowered significantly after 7 weeks of intense sprint training. Furthermore, Tullson et al. (1995) reported a decrease in total purines between the end of an exercise period and 3 minutes into recovery, indicating that there are rapid changes in total adenine nucleotide pool levels due to degradation and purine efflux.

Researchers have suggested that ATP synthesis may be a gradual process, because the total adenine nucleotide pool is replenished through the slow *de novo* synthesis (Sheehan and Tully, 1983; Tullson and Terjung, 1991; Hellsten et al., 1999) (Figure 1).

The delayed recovery of ATP and the total adenine nucleotide pool is likely explained by the lack of the availability of 5-phosphoribosyl-1-pyrophosphate (PRPP), the rate limiting factor in adenine nucleotide synthesis and salvage (Henderson and Khoo, 1965; Manfredi and Holmes, 1985; Mauser at al., 1985; Zimmer et al., 1990). Several research papers indicate that PRPP availability limits adenine nucleotide synthesis by both the *de novo* and salvage pathways (Zimmer, 1980; Zimmer and Ibel, 1983). Ribose-5-phosphate (R-5-P) is generated from glucose via the pentose phosphate pathway (PPP) (Figure 1). The R-5-P is then converted to PRPP, which is used in the *de novo* synthesis of ATP.

Glucose-6-phosphate dehydrogenase (G-6-PDH) is the rate-limiting enzyme during the production of R-5-P; and later, of PRPP via the PPP (Eggleston and Krebs, 1974; Wood, 1985; Klietzien et al., 1994; Martini and Ursini, 1996; Tian et al., 1998; Tuttle et al., 2000). There are two ways that PRPP levels can be increased. The first is,

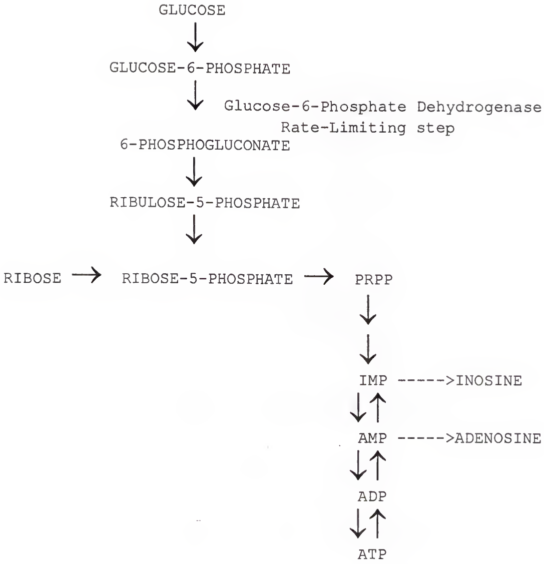

Figure 1. The pentose phosphate pathway (PPP) and the role of ribose in the *de novo* synthesis of ATP (PRPP = 5-phosphoribosyl-1-pyrophosphate. IMP = inosine monophosphate. AMP = adenosine monophosphate. ADP = adenosine diphosphate. ATP = adenosine triphosphate.)

by stimulating the PPP, which would increase the supply of R-5-P to be used for PRPP production The second is by providing other substances that are likely to be converted to PRPP. Several researchers have noted that pentoses (i.e., ribose) bypass the PPP and contribute to the formation of PRPP (Horecker et al., 1956; McCormick and Touster, 1957; Segal and Foley, 1958; Hauschildt and Watts, 1976). This metabolic basis for the effectiveness of ribose is not species specific. The enzyme G-6-PDH is the rate-limiting enzyme in the heart and skeletal muscle for rats, dogs, swine, and humans (Zimmer and Schad, 1984).

Ribose supplementation could, therefore, directly increase PRPP concentrations (Harmsen et al., 1984; Zimmer, 1998; Barsotti and Ipata, 2002). The elevated PRPP levels could then be used to replenish skeletal muscle adenine nucleotide pools (particularly ATP) which could then contribute to a better performance by the exercising horse.

Ribose Studies

Studies have shown that ribose infusion to isolated skeletal muscle increases the recovery of ATP (Tullson and Terjung, 1991; Brault and Terjung, 2001; Zarzeczny et al., 2001). Tullson and Terjung (1991) measured the *de novo* synthesis rates of the total adenine nucleotide pool in relaxed and contracting muscle by using a perfused rat hindquarter preparation. The authors reported that adding 5 mM ribose to the perfusate increased the rate of the *de novo* adenine nucleotide synthesis by 3.4 to 4.3 times compared to the rate measured in sedentary animals that were not perfused with ribose. They also reported that ribose supplementation significantly increased ATP synthesis in resting muscle (Tullson and Terjung, 1991).

Brault and Terjung (2001) studied the rates of purine salvage of adenine and hypoxanthine into the total adenine nucleotide pool by using an isolated perfused rat hindquarter preparation. The authors reported that the purine salvage rates were significantly greater for adenine than for hypoxanthine, and that addition of ribose to the perfusion medium resulted in a significant increase in the salvage of adenine and hypoxanthine.

Zarzeczny et al. (2001) studied the influence of ribose supplementation on skeletal muscle adenine salvage rates during recovery from intense contractions and subsequent muscle performance by using a perfused rat hindquarter preparation. The authors stated that 3 minutes of tetanic contractions decreased the ATP content of the calf muscles by about 50% and produced an equimolar increase in IMP. During the first hour of recovery, the adenine salvage rates were increased five to seven times by ribose perfusion. Despite the marked increase in the rates of adenine salvage in the presence of ribose, there was no significant impact on the total adenine nucleotide content within the muscle. The authors reported that ribose infusion did not result in significant changes in the total adenine nucleotide content because, despite the high rates of adenine salvage (200-250 nmol per hour per gram) observed in this study, these rates were still relatively small when compared to the large pool of adenine nucleotides in the muscle (8,000-9,000 nmol per gram). The authors concluded that 1 hour of recovery might have been inadequate to detect differences in the total adenine nucleotide pool (Zarzeczny et al., 2001).

Researchers have attempted to document the effects of oral ribose supplementation as an ergogenic aid in exercising humans, but the results have been equivocal. Muscle power output was measured during dynamic knee extensions on an isokinetic dynamometer before and after a 6-day training period in conjunction with either ribose supplementation (4 doses per day at 4 grams per dose for 10 days) or placebo (Op 'T Eijnde et al., 2001). Knee extension power output was higher in the post treatment than the pre treatment, but was similar between the placebo and ribose treatment groups. Also, there were no significant differences for blood lactic acid or ammonia between the two treatment groups. In phase II of the experiment (Op 'T Eijnde et al., 2001), needle biopsies were collected before, 3 minutes, and 24 hours after a similar exercise protocol to phase I. The subjects had received either ribose or placebo supplement for 3 days before the biopsies. Three minutes after the exercise bout, ATP content was significantly decreased in both treatment groups. After 24 hours of recovery, the ATP content increased in both the placebo and ribose treatment groups, but was still significantly lower than the corresponding pre-exercise levels. The authors concluded that oral ribose supplementation did not impact on post-exercise muscle ATP recovery or exercise performance as studied under their experimental conditions (Op 'T Eijnde et al., 2001).

Kreider at al. (2003) investigated the effects of ribose supplementation (5 grams of ribose twice daily for 5 days) in trained males performing two anaerobic sprint tests on a cycle ergometer. No significant differences were observed in peak power, average power, torque, fatigue index, or serum lactic acid, ammonia, glucose, or uric acid between the two treatment groups. The authors concluded that the results obtained indicate that oral ribose supplementation did not affect anaerobic exercise capacity or metabolic markers in trained subjects as evaluated by their protocol.

Trappe (1999) investigated the effects of ribose supplementation (10 grams of ribose 3 times per day for 6 days) on the recovery of the adenine nucleotide pool and power output during cycle sprints. The author reported that the ribose treatment group had increased power output and quicker recovery of the adenine nucleotide pool levels compared to the controls. However, an experimental weakness of this study was that there were only two subjects per treatment group and each subject received only one treatment.

Gross et al. (1991) reported that acute ingestion of ribose (2 grams of ribose every 5 minutes for 30 minutes) during bicycle exercise blunted the increase in plasma hypoxanthine levels, suggesting a reduction in ATP degradation.

Gallagher et al. (2001) investigated the effects of ribose supplementation (20 grams of ribose per day for 11 days) on adenine nucleotide levels in human skeletal muscle after high-intensity cycle exercise. Muscle biopsies were performed before and after exercise; and were analyzed for total adenine nucleotide levels. At 65 hours after the exercise bout, the ribose treatment group returned to pre-exercise total adenine nucleotide levels, while the placebo treatment group remained 23% below the pre-exercise levels ($P < 0.05$). The authors concluded that ribose supplementation may attenuate the decrease in the total adenine nucleotide pool after high-intensity cycle exercise; and that ribose supplementation may be beneficial in short-term replenishment of the total adenine nucleotide pool.

Raue et al. (2001) reported that males supplemented with 20 grams of ribose per day for 11 days had significantly greater peak power output at the last of a series of sprint sessions, while no differences were noted in the placebo treatment group. The authors concluded that ribose supplementation may provide an ergogenic benefit with high intensity cycle exercise training.

In another study by Van Gammeren et al. (2002), ribose supplementation (10 grams of ribose per day for 4 weeks) resulted in a significant pre-treatment to post-treatment increase in total work as measured by work performed on the bench press. The placebo treatment group did not show a significant pre-treatment to post-treatment increase in total work. Ribose supplementation also resulted in a significant increase in 1-repetition maximum bench-press strength, whereas the placebo treatment group did not.

Berardi and Ziegenfuss (2003) investigated the effects of ribose supplementation (8 grams of ribose four times per day consumed in 36 hours) on cycle sprint exercise (six 10 second sprints with 60 second rest periods between sprints). Ribose supplementation led to significant increases in mean power and peak power in sprint 2; and higher (although not significant) absolute values in the other five sprints. The authors concluded that ribose supplementation may be beneficial during anaerobic cycle sprinting.

The results from these studies suggest that oral ribose supplementation may, in some cases, result in metabolic changes and improve performance. However, other studies did not find benefits from oral ribose supplementation. The differences observed may in part be attributed to the differences in the training protocols, exercise tests, or the amount of ribose supplementation used.

Summary

Ribose is a simple pentose sugar found in every living cell, and ribose can be used by the body to build and restore energy in the form of ATP. ATP and the total adenine nucleotide pool levels decrease during intense physical exercise and their replenishment appears to be a slow process. Some reports have indicated that the administration of ribose can increase power output in athletes and can improve the ability of skeletal muscle to restore ATP levels after high-intensity exercise. Ribose is rapidly absorbed from the gastrointestinal tract and is very well tolerated (Gross et al., 1989; Gross et al., 1991). After absorption, ribose is rapidly and extensively metabolized via the PPP (Segal and Foley, 1958); and does not result in any abnormal hematological or hepatological variable changes (Frelich and Seifert, 2001). As important as ribose appears to be, there is no known metabolically significant food source of ribose. Because of economic constraints, ribose has not been available commercially until recently. Modern manufacturing processes enabled ribose to be obtained from yeast (DeWulf and Vandamme, 1997a; DeWulf and Vandamme, 1997b), making it available to food, nutraceutical, pharmaceutical, and health-care industries.

Rationale

Research indicates that ribose supplementation can be used to restore cardiac ATP levels after an ischemic attack. Recently, attempts have been made to document whether ribose supplementation can maintain or increase the ATP levels in exercising skeletal muscle; and whether ribose can be used as an ergogenic aid during exercise. The results from a limited number of human studies have been equivocal. To date, there is no known investigation on the effects of ribose supplementation in exercising horses.

Objectives

The current study was designed to investigate the effects of ribose supplementation on selected metabolic parameters and performance in Thoroughbred horses performing a standardized exercise test (SET). The objective of the current study was to determine whether oral ribose supplementation would influence the metabolic parameters measured. Another objective of the current study was to investigate whether ribose supplementation would improve performance in Thoroughbred horses performing a SET.

Hypotheses

The null hypothesis of the current study was that there would be no difference in the metabolic parameters measured between the two treatments groups (Ho: $\mu_P = \mu_R$), where μ_P = true mean concentration of metabolites in the placebo treatment group, and μ_R = true mean concentration of metabolites in the ribose treatment group. The alternative hypothesis was that the parameters would be different between the two treatment groups (Ha: $\mu_P \neq \mu_R$). Also, another null hypothesis tested was that the performance between the two treatment groups would be the same. The alternative hypothesis tested was that the performance would be different between the two treatment groups.

CHAPTER 2
MATERIALS AND METHODS

Experiment 1

Training

Six Thoroughbred geldings were habituated to running on a high speed treadmill and their workload was gradually increased. The first 2 days the horses trotted at 4 m/s for 0.6 km on a flat surface, galloped at 8 m/s for 1.5 km on a flat surface, and then cooled down by trotting at 4 m/s for 0.4 km on a flat surface. The gallop distance was increased by 0.5 km every 2 days. By the end of 2 weeks the horses were performing the following exercise: warm up by trotting at 4 m/s for 0.6 km on a flat surface, gallop at 8 m/s for 3.0 km on a flat surface, and then cool down by trotting at 4 m/s for 0.4 km on a flat surface. The horses were then trained 4 times per week. On Monday and Thursday the horses exercised with the same intensity as described above. On Tuesday and Friday the horses exercised as above, but they galloped 3.0 km at 6° incline. On Wednesday, Saturday, and Sunday the horses did not exercise on the treadmill. The training period lasted 12 weeks.

Diet

The horses were fed a concentrate and coastal bermuda grass hay (Table 1) twice daily, once in the morning and once in the afternoon. The horses were weighed every 2 weeks for the duration of the study, and a body condition score between 1 and 9 was assigned according to Henneke et al. (1983). The amount of concentrate fed was adjusted to maintain a desired body condition score of 5. The horses had *ad libitum* access to salt blocks and clean water.

Facilities

The horses were housed at the University of Florida Department of Animal Sciences' Horse Nutrition Barn in individual stalls to permit control of feed intake. Training and SET were performed at the College of Veterinary Medicine, University of Florida, in a climate controlled room equipped with a treadmill (SATO II, Equine Dynamics, Inc., Lexington, KY).

Randomization

After 12 weeks of training the horses performed a SET and the VO_{2max} of each horse was recorded. The horses were ranked from 1 to 6, horse 1 being the one with the highest

Table 1. Concentrate composition (Experiment 1, Experiment 2, and Experiment 3)

Ingredient	Percent (%)
Corn, cracked	34.25
Oats, crimped	26.5
Soybean meal, 48% CP	10
Wheat bran	10
Molasses, blackstrap	8
Alfalfa meal, 17% CP	7.5
Limestone, ground	1.5
Mono dical	0.8
Salt	0.75
Vitamin premix[a]	0.3
Vitamin E[b]	0.15
Lysine 98%	0.05
Luprosil[c]	0.1
Trace Mineral premix[d]	0.1

[a] Provided 4,400,000 IU vitamin A, 440,000 IU vitamin D, and 35,200 IU vitamin E/kg premix

[b] Provided 44,200 IU Vit E (DL-α-tocopherol acetate)/kg premix

[c] Mold inhibitor

[d] Provided 14,300 mg Cu, 40,000 mg Zn, 28,000 mg Fe, 28,000 mg Mn, 80 mg Co, 160 mg I, and 160 mg Se/kg premix

VO_{2max} and horse 6 being the one with the lowest VO_{2max}. Then, horses 1 and 2 were assigned to cluster 1, horses 3 and 4 were assigned to cluster 2, and horses 5 and 6 were assigned to cluster 3. One horse from each of the three clusters was then randomly assigned to one of the two treatment groups (placebo or ribose), and the remaining horse in each cluster was assigned to the other treatment group.

Treatment Groups

The two treatment groups were placebo and ribose. The placebo treatment group received 0.14 grams of glucose per kg BW top dressed on their feed daily. The ribose treatment group received 0.14 grams of ribose per kg BW top dressed on their feed daily. The amount of glucose or ribose supplement was divided to two equal portions and was administered at the morning and afternoon feedings. The horses received their assigned supplement for 2 weeks while continuing to exercise on the treadmill four times per week, with the same duration and intensity as the training period. After 2 weeks of supplementation a SET was performed. After 1 week washout period the horses switched treatment groups, and the horses received that supplement for 2 weeks before a second SET was performed.

Response Variable Measurements and Laboratory Analyses

Within 1 hour prior to the SET, the left jugular vein was cannulated with a 14 gauge X 14 cm catheter for venous blood sample collection. Blood was collected at 15 and 0 minutes pre-exercise, at 8 m/s, 9 m/s, 10 m/s, 11 m/s, 12 m/s, 13 m/s, and at 15 and 30 minutes post-exercise. If a horse did not exercise for 40 seconds at a given speed, blood was not collected.

Blood for ammonia determination was collected in sodium heparin tubes, and immediately deproteinized using 10% sodium tungstate and 1N sulfuric acid. The deproteinized supernatants were frozen at -80° C. Blood ammonia was determined by a spectrophotometric technique described by McCullough (1967) within 24 hours of sample collection.

Blood for plasma lactic acid determination was collected in potassium oxalate/sodium fluoride tubes, and kept on ice until centrifuged within 30 minutes after collection. Plasma was separated and stored at -80° C. Plasma lactic acid was determined by a spectrophotometric technique utilizing a kit (#735-10) from Sigma Chemicals, St. Louis, MO.

Blood for plasma glucose determination was collected in potassium oxalate/sodium fluoride tubes, and it was kept on ice until centrifuged within 30 minutes after collection. Plasma was separated and stored at -80° C. Plasma glucose concentrations were determined by a spectrophotemetric technique utilizing a kit (#315-100) from Sigma Chemicals, St. Louis, MO.

Blood for ATP determination was collected in sodium heparin tubes and 1 mL of blood was immediately deproteinized with 1 mL 12% trichloroacetic acid (TCA). After centrifugation, the supernatant was carefully aspirated, transferred to a micro-vial, and kept on ice until analyzed for ATP concentrations within 5 hours of collection by using a spectrophotemetric technique utilizing a kit (#366) from Sigma Chemicals, St. Louis, MO.

Venous blood was collected in heparinized syringes and analyzed for pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^- at 15 minutes pre-exercise, at the end of the SET, and at 30 minutes post-exercise by using an automated analyzer (Instrumentation Laboratory, Lexington, MA).

Muscle punch biopsies (4mm in diameter), from the *gluteus medius*, were collected at 15 minutes pre-exercise, immediately post-exercise, and 48 hours post-exercise.

Immediately after removal, the muscle biopsy was dissected into pieces and the samples were frozen in liquid nitrogen within two minutes of sampling. The samples were stored at -80^0C until subsequent analyses.

Muscle glycogen concentrations were determined by the spectrophotometric technique described by Lo et al. (1970).

Individual frozen samples were freeze-dried, dissected free of blood, connective tissue and fat, then extracted in perchloric acid, followed by potassium hydrogen carbonate neutralization (Harris et al., 1974). The extract was analyzed for lactic acid by a spectrophotometric technique by utilizing a kit (#735-10) from Sigma Chemicals, St. Louis, MO.

During the SET, oxygen intake (VO_2) was measured every 10 seconds by using a paramagnetic oxygen sensor (Columbus Instruments, Columbus, OH).

Heart rate was recorded at 0 minutes pre-exercise and at 8 m/s, 9 m/s, 10 m/s, 11 m/s, 12 m/s, and 13 m/s (Equine Performance Technology, Reliance, TN).

Performance was recorded as total run time in seconds beginning from 8 m/s until the horse could no longer stay in place on the treadmill despite urging.

Standardized Exercise Test (SET)

Warm up consisted of a trot at 4 m/s for 0.6 km. The treadmill speed was then increased to 8 m/s and the treadmill surface was elevated to 6^0 incline. The horses exercised for one minute at each of the following speeds, 8, 9, 10, 11, 12, and 13 m/s, until they failed to stay in place on the treadmill despite urging. The treadmill was then lowered to a flat surface and the horses were taken off the treadmill and hand walked.

Statistical Analysis

Data are shown as mean ± standard deviation. Data were analyzed using repeated measures and the pair-difference t-test procedure of SAS (1989). All results with $P < 0.05$ were considered statistically significant.

Experiment 2

Training

Five of the six trained Thoroughbred geldings from Experiment 1 were used in Experiment 2. Horse D was withdrawn from Experiment 2 because of a foot abscess.

Diet

The horses were fed the same diet (Table 1) as in Experiment 1.

Facilities

The animals were housed in the same facilities as in Experiment 1.

Randomization

The horses were randomized to one of two treatment groups by flipping a coin.

Treatment Groups

The two treatment groups were placebo and ribose. One hour before a SET, the placebo treatment group (n = 2) received 3 L of water, and the ribose treatment group (n = 3) received 250 g of ribose dissolved in 3 L of water via a nasogastric tube. After a two week washout period the horses switched treatment groups, and another SET was performed and data collection was repeated.

Response Variable Measurements and Laboratory Analyses

The same procedures were followed as Experiment 1. Blood for ATP analyses and muscle punch biopsies were not obtained from Experiment 2.

Standardized Exercise Test (SET)

The same SET was used as in Experiment 1.

Statistical Analysis

The same statistical analysis was used as in Experiment 1.

Experiment 3

Training

Eight Thoroughbred geldings were trained according to the protocol from Experiment 1. The training period lasted 11 weeks.

Diet

The horses were fed the same diet (Table 1) as in Experiment 1.

Facilities

The animals were housed in the same facilities as in Experiment 1.

Randomization

After eleven weeks of conditioning the horses performed a SET and the performance (measured as total seconds of exercise until they failed to stay in place on the treadmill despite urging) of each horse was recorded. Then the same randomization process was used as in Experiment 1, using performance instead of VO_{2max}.

Treatment Groups

The two treatment groups were placebo and ribose. The placebo treatment group received 0.30 grams of glucose per kg BW top dressed on their feed daily. The ribose treatment group received 0.30 grams of ribose per kg BW top dressed on their feed daily. The amount of glucose or ribose supplement was divided to two equal portions and was administered at the morning and afternoon feedings. The horses were fed according to their assigned treatment for 2 weeks while continuing to exercise on the treadmill four times per week, with the same duration and intensity as the training period.

Response Variable Measurements and Laboratory Analyses

A SET was performed after 2 weeks of supplementation. The same procedures as in Experiment 1 were used. However, blood ammonia, plasma lactic acid, plasma glucose, VO_2, heart rate, and performance were the only parameters recorded. Blood was not collected at 15 minutes pre-exercise. After a 10 day washout period the horses switched treatment groups, and the horses received that supplement for 2 weeks before a second SET was performed, and same data were recorded.

Standardized Exercise Test (SET)

The same SET was used as in Experiment 1.

Statistical Analysis

The same statistical analysis was used as in Experiment 1.

CHAPTER 3
RESULTS

Experiment 1

General

The nutrient analyses of the concentrate and hay are shown in Table 2. The horses were fed 2.4 ± 0.2 kg concentrate and 2.8 ± 0.1 kg hay. The age of the six horses are shown in Table 3. The body weight and body condition score (BCS) of the six horses are shown in Table 4.

Performance

The duration of the SET of each horse is shown in Table 5. There were no differences between treatment groups for total run time ($P > 0.05$).

Blood Ammonia

Peak blood ammonia occurred at 15 minutes post-exercise and was 339.1 ± 84.3 µmol/L in the placebo treatment group, and 310.1 ± 106.5 µmol/L in the ribose treatment group ($P > 0.05$). At 30 minutes post-exercise the placebo treatment group had higher blood ammonia than the ribose treatment group (280.8 ± 98.8 vs 246.2 ± 91.8

Table 2. Nutrient analyses of the concentrate and hay[a] (Experiment 1 and 2)

Nutrient	Concentrate	Hay
Crude Protein (g/kg)	159.1	69.3
Acid detergent fiber (g/kg)	146.1	320.8
Neutral detergent fiber (g/kg)	296.6	774.2
Ether extract (g/kg)	34.5	18.6
Calcium (g/kg)	11.8	3.6
Phosphorus (g/kg)	6.8	1.8

[a] Dry matter basis

Table 3. Age of six horses (Experiment 1 and 2)

Horse	Age (years)
A	12
B	7
C	4
D	3
E	3
F	3

Table 4. Weight and body condition score (BCS) of horses (Experiment 1)

Horse A

Time	Weight (kg)	BCS
0 weeks	517	4.5
2 weeks	528	4.5
4 weeks	494	4.5
6 weeks	538	5.0
8 weeks	540	5.0
10 weeks	520	5.0
12 weeks	508	5.0
1st SET	499	4.5
2nd SET	495	4.5

Horse B

Time	Weight (kg)	BCS
0 weeks	578	4.5
2 weeks	599	5.0
4 weeks	584	5.0
6 weeks	603	5.5
8 weeks	590	5.5
10 weeks	583	5.0
12 weeks	565	5.0
1st SET	556	4.5
2nd SET	558	4.5

Horse C

Time	Weight (kg)	BCS
0 weeks	510	4.5
2 weeks	531	4.5
4 weeks	519	4.5
6 weeks	535	5.0
8 weeks	531	5.0
10 weeks	518	5.0
12 weeks	502	4.5
1st SET	488	4.5
2nd SET	486	4.5

Table 4. Continued

	Horse D	
Time	Weight (kg)	BCS
0 weeks	499	4.5
2 weeks	479	4.5
4 weeks	490	4.5
6 weeks	513	5.0
8 weeks	513	5.0
10 weeks	508	5.0
12 weeks	479	4.5
1st SET	481	4.5
2nd SET	474	4.5

	Horse E	
Time	Weight (kg)	BCS
0 weeks	530	5.0
2 weeks	513	5.0
4 weeks	508	5.0
6 weeks	526	5.5
8 weeks	518	5.0
10 weeks	513	5.0
12 weeks	490	5.0
1st SET	477	4.5
2nd SET	463	4.5

	Horse F	
Time	Weight (kg)	BCS
0 weeks	515	5.0
2 weeks	513	5.0
4 weeks	513	5.0
6 weeks	526	5.0
8 weeks	513	5.0
10 weeks	518	5.0
12 weeks	504	4.5
1st SET	486	4.5
2nd SET	488	4.5

Table 5. Influence of ribose supplementation on the duration of exercise during a standardized exercise test (SET) on the treadmill (Experiment 1)

Horse	Placebo		Ribose	
	Speed[a] (m/s)	Time[b] (s)	Speed[a] (m/s)	Time[b] (s)
A	11	45	11	45
B	12	10	12	05
C	11	60	11	45
D	12	30	12	25
E	12	60	12	60
F	12	60	13	05

[a] Highest speed attained

[b] Length of time at the designated speed at the end of the SET

μmol/L, $P < 0.05$). No other differences ($P > 0.05$) were observed for blood ammonia between treatments (Table 6).

Plasma Lactic Acid

There were no differences ($P > 0.05$) for plasma lactic acid between treatment groups (Table 7). Peak plasma lactic acid occurred at 15 minutes post-exercise and was 24.5 ± 2.2 mmol/L in the placebo treatment group and 23.0 ± 4.3 mmol/L in the ribose treatment group.

Plasma Glucose

There were no differences ($P > 0.05$) for plasma glucose between treatment groups (Table 8). Peak plasma glucose occurred at 15 minutes post-exercise and was 7.26 ± 1.2 mmol/L in the placebo treatment group, and 7.79 ± 0.9 mmol/L in the ribose treatment group.

Blood ATP

There were no differences ($P > 0.05$) for blood ATP between treatment groups (Table 9). Peak blood ATP occurred at the last exercise step and was 28.0 ± 3.1 μmol/dL in the placebo treatment group, and 29.7 ± 5.4 μmol/dL in the ribose treatment group.

Blood Gasses and Electrolytes

The results for pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^- obtained at 15 minutes pre-exercise, at the end of the SET,

Table 6. Influence of ribose supplementation on blood ammonia (µmol/L) in exercising horses (Experiment 1)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	109.2 ± 12.7	114.4 ± 18.7	0.58
0 minutes pre	105.0 ± 13.6	104.6 ± 6.9	0.96
8 m/s	110.2 ± 23.4	103.3 ± 11.0	0.60
9 m/s	106.0 ± 6.2	101.5 ± 9.4	0.09
10 m/s	127.2 ± 30.9	122.2 ± 17.6	0.50
11 m/s	168.2 ± 87.8	132.2 ± 19.4	0.76
12 m/s	161.0 ± 38.3[a]	148.8 ± 27.2[a]	0.36
15 minutes post	339.1 ± 84.3	310.1 ± 106.5	0.06
30 minutes post	280.8 ± 98.8	246.2 ± 91.8	0.02

[a] Only two horses reached this speed step

Table 7. Influence of ribose supplementation on plasma lactic acid (mmol/L) in exercising horses (Experiment 1)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	0.8 ± 0.1	0.9 ± 0.1	0.33
0 minutes pre	0.8 ± 0.1	0.8 ± 0.2	0.98
8 m/s	5.3 ± 1.4	4.4 ± 1.3	0.19
9 m/s	9.8 ± 3.8	10.0 ± 5.5	0.93
10 m/s	14.2 ± 5.6	13.3 ± 4.5	0.44
11 m/s	20.5 ± 7.3	15.8 ± 4.8	0.18
12 m/s	21.0 ± 2.9[a]	20.6 ± 3.6[a]	0.51
15 minutes post	24.5 ± 2.2	23.0 ± 4.3	0.23
30 minutes post	18.6 ± 4.3	15.4 ± 3.5	0.07

[a] Only two horses reached this speed step

Table 8. Influence of ribose supplementation on plasma glucose (mmol/L) in exercising horses (Experiment 1)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	5.81 ± 1.05	6.44 ± 1.15	0.45
0 minutes pre	5.74 ± 1.01	6.54 ± 1.22	0.18
8 m/s	5.88 ± 1.14	6.49 ± 0.97	0.24
9 m/s	6.27 ± 1.43	6.81 ± 0.74	0.30
10 m/s	6.69 ± 1.58	6.98 ± 1.09	0.62
11 m/s	6.97 ± 1.69	7.12 ± 1.63	0.25
12 m/s	7.12 ± 0.76[a]	8.70 ± 0.39[a]	0.30
15 minutes post	7.26 ± 1.16	7.79 ± 0.89	0.22
30 minutes post	6.79 ± 1.20	7.36 ± 1.22	0.28

[a] Only two horses reached this speed step

Table 9. Influence of ribose supplementation on blood ATP (μmol/dL) in exercising horses (Experiment 1)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	19.7 ± 2.7	19.7 ± 2.3	0.98
0 minutes pre	20.9 ± 2.8	20.6 ± 1.5	0.84
8 m/s	24.4 ± 4.5	24.9 ± 3.8	0.84
9 m/s	25.3 ± 5.8	23.6 ± 5.3	0.67
10 m/s	27.6 ± 4.0	28.9 ± 5.1	0.21
11 m/s	28.0 ± 3.1	29.7 ± 5.4	0.54
12 m/s	26.9 ± 5.8[a]	29.6 ± 3.5[a]	0.35
15 minutes post	24.3 ± 2.5	24.8 ± 4.1	0.69
30 minutes post	22.7 ± 2.9	22.5 ± 2.6	0.78

[a]Only two horses reached this speed step

and at 30 minutes post-exercise are shown in Table 10. At 15 minutes pre-exercise, horses in the ribose treatment group had lower K^+ compared to placebo treatment group ($P < 0.05$). No other differences ($P > 0.05$) were observed between treatment groups.

Muscle Glycogen

At rest, the placebo treatment group had 26.7 ± 2.3 and the ribose treatment group had 25.2 ± 0.7 mmol glycocyl units/kg wet muscle weight ($P > 0.05$). After the SET, the placebo treatment group had 18.1 ± 2.6 and the ribose treatment group had 16.1 ± 1.2 mmol glycocyl units/kg wet muscle weight ($P > 0.05$). At 48-hour post-exercise, the placebo treatment group had 27.2 ± 2.5 and ribose treatment group had 26.3 ± 1.9 mmol glycocyl units/kg wet muscle weight ($P > 0.05$).

Muscle Lactic Acid

At rest, the placebo treatment group had 2.0 ± 1.2 and the ribose treatment group had 2.3 ± 0.8 mmol lactic acid/kg dry muscle weight ($P > 0.05$). After the SET, the placebo treatment group had 17.0 ± 4.5 and the ribose treatment group had 18.0 ± 3.3 mmol lactic acid/kg dry muscle weight ($P > 0.05$).

Table 10. Influence of ribose supplementation on venous blood pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^- in exercising horses (Experiment 1)

Item	Placebo	Ribose	p-value
pH			
15 minutes pre	7.412 ± 0.012	7.412 ± 0.012	0.98
end of SET	6.965 ± 0.034	7.003 ± 0.043	0.11
30 minutes post	7.257 ± 0.064	7.299 ± 0.060	0.08
K^+ (mmol/L)			
15 minutes pre	3.7 ± 0.2	3.5 ± 0.2	0.02
end of SET	7.3 ± 0.6	6.8 ± 0.7	0.33
30 minutes post	3.8 ± 0.5	3.8 ± 0.3	0.78
Na^+ (mmol/L)			
15 minutes pre	137.7 ± 1.9	137.7 ± 2.0	1.00
end of SET	151.5 ± 2.9	150.7 ± 5.3	0.72
30 minutes post	140.2 ± 2.9	139.2 ± 3.2	0.52
Ca^+ (mmol/L)			
15 minutes pre	1.43 ± 0.06	1.44 ± 0.07	0.74
end of SET	1.43 ± 0.23	1.47 ± 0.09	0.70
30 minutes post	1.25 ± 0.18	1.35 ± 0.04	0.25
pCO_2 (mmHg)			
15 minutes pre	52.5 ± 2.7	51.1 ± 4.5	0.53
end of SET	112.9 ± 15.6	99.1 ± 31.6	0.25
30 minutes post	33.4 ± 4.4	34.8 ± 5.1	0.30
HCO_3^- (mmol/L)			
15 minutes pre	33.7 ± 1.0	32.9 ± 2.7	0.41
end of SET	26.2 ± 4.8	24.6 ± 5.7	0.34
30 minutes post	15.4 ± 3.7	17.6 ± 4.2	0.10

Volume of Oxygen Consumption

There was no difference between the placebo and ribose treatment groups for VO_{2max} ($P > 0.05$). VO_{2max} was 172 ± 35 ml/kg/min in the placebo treatment group, and 158 ± 14 ml/kg/min in the ribose treatment group.

Heart Rate

Heart rate was not different between treatment groups at rest or during exercise ($P > 0.05$). Peak heart rate for each horse was recorded during the last exercise step, and ranged from 197 to 220 in the placebo treatment group, and from 199 to 219 in the ribose treatment group.

Experiment 2

General

The nutrient analyses of the concentrate and hay are shown in Table 2. The horses were fed 2.6 ± 0.2 kg concentrate and 2.8 ± 0.2 kg hay. The body weight and BCS of the six horses are shown in Table 11.

Performance

The duration of the SET of each horse is shown in Table 12. There were no differences between treatment groups for total run time ($P > 0.05$).

Table 11. Weight and body condition score (BCS) of horses (Experiment 2)

	Horse A	
Time	Weight (kg)	BCS
1st SET	490	4.5
2nd SET	496	4.5

	Horse B	
Time	Weight (kg)	BCS
1st SET	549	4.5
2nd SET	550	4.5

	Horse C	
Time	Weight (kg)	BCS
1st SET	477	4.5
2nd SET	484	4.5

	Horse D	
Time	Weight (kg)	BCS
1st SET	–[a]	–[a]
2nd SET	–[a]	–[a]

	Horse E	
Time	Weight (kg)	BCS
1st SET	459	4.5
2nd SET	455	4.5

	Horse E	
Time	Weight (kg)	BCS
1st SET	484	4.5
2nd SET	473	4.5

–[a]Horse D withdrawn

Table 12. Influence of ribose supplementation on the duration of exercise during a standardized exercise test (SET) on the treadmill (Experiment 2)

Horse	Placebo		Ribose	
	Speed[a] (m/s)	Time[b] (s)	Speed[a] (m/s)	Time[b] (s)
A	12	10	11	60
B	11	55	11	55
C	11	50	12	20
D	–[c]	–[c]	–[c]	–[c]
E	12	60	13	05
F	12	60	13	05

[a] Highest speed attained

[b] Length of time at the designated speed at the end of the SET

[c] Horse D withdrawn

Blood Ammonia

No differences ($P > 0.05$) were observed for blood ammonia between treatment groups (Table 13). Peak blood ammonia occurred at 15 minutes post-exercise and was 213.9 ± 68.1 µmol/L in the placebo treatment group, and 235.1 ± 95.5 µmol/L in the ribose treatment group.

Plasma Lactic Acid

There were no differences ($P > 0.05$) for plasma lactic acid between treatment groups (Table 14). Peak plasma lactic acid occurred at 15 minutes post-exercise and was 20.3 ± 3.1 mmol/L in the placebo treatment group, and 20.8 ± 4.0 mmol/L in the ribose treatment group.

Plasma Glucose

There were no differences ($P > 0.05$) for plasma glucose between treatment groups (Table 15). Peak plasma glucose occurred at 15 minutes post-exercise and was 7.00 ± 1.31 mmol/L in the placebo treatment group, and 7.09 ± 0.59 mmol/L in the ribose treatment group.

Blood Gasses and Electrolytes

The results for pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^- obtained at 15 minutes pre-exercise, at the end of the SET, and at 30 minutes post-exercise are shown in Table 16. No

Table 13. Influence of ribose supplementation on blood ammonia (μmol/L) in exercising horses (Experiment 2)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	99.5 ± 7.4	97.2 ± 3.5	0.61
0 minutes pre	93.2 ± 14.3	91.1 ± 11.5	0.86
8 m/s	96.0 ± 12.0	95.1 ± 8.5	0.90
9 m/s	96.3 ± 11.5	93.8 ± 11.6	0.95
10 m/s	106.8 ± 16.5	104.0 ± 10.1	0.78
11 m/s	134.0 ± 17.2	133.0 ± 35.2	0.92
12 m/s	119.4 ± 0.1[a]	123.0 ± 15.8[a]	0.80
15 minutes post	213.9 ± 68.1	235.1 ± 95.5	0.34
30 minutes post	158.7 ± 56.5	179.8 ± 84.6	0.26

[a] Only two horses reached this speed step

Table 14. Influence of ribose supplementation on plasma lactic acid (mmol/L) in exercising horses (Experiment 2)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	0.7 ± 0.1	0.7 ± 0.2	0.88
0 minutes pre	0.7 ± 0.2	0.7 ± 0.1	0.97
8 m/s	4.3 ± 0.5	4.0 ± 0.7	0.23
9 m/s	8.6 ± 2.6	7.3 ± 1.5	0.19
10 m/s	11.9 ± 3.4	10.7 ± 2.8	0.25
11 m/s	17.2 ± 5.1	16.4 ± 5.6	0.63
12 m/s	17.9 ± 1.1[a]	17.1 ± 0.9[a]	0.64
15 minutes post	20.3 ± 3.1	20.8 ± 4.0	0.73
30 minutes post	14.2 ± 3.8	14.1 ± 4.4	0.90

[a] Only two horses reached this speed step

Table 15. Influence of ribose supplementation on plasma glucose (mmol/L) in exercising horses (Experiment 2)

Time and speed	Placebo	Ribose	p-value
15 minutes pre	5.77 ± 0.92	5.26 ± 0.41	0.27
0 minutes pre	5.49 ± 0.58	5.15 ± 0.40	0.27
8 m/s	5.56 ± 0.72	5.22 ± 0.39	0.21
9 m/s	5.81 ± 0.98	5.37 ± 0.42	0.25
10 m/s	5.76 ± 0.91	5.55 ± 0.40	0.61
11 m/s	5.79 ± 1.06	5.83 ± 0.54	0.91
12 m/s	5.61 ± 0.69[a]	5.99 ± 0.62[a]	0.09
15 minutes post	7.00 ± 1.31	7.09 ± 0.59	0.86
30 minutes post	6.68 ± 0.97	6.78 ± 0.78	0.82

[a] Only two horses reached this speed step

Table 16. Influence of ribose supplementation on venous blood pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^- in exercising horses (Experiment 2)

Item	Placebo	Ribose	p-value
pH			
15 minutes pre	7.415 ± 0.014	7.399 ± 0.006	0.06
end of SET	7.097 ± 0.051	7.052 ± 0.088	0.33
30 minutes post	7.321 ± 0.058	7.319 ± 0.060	0.89
K^+ (mmol/L)			
15 minutes pre	3.6 ± 0.4	3.4 ± 0.2	0.18
end of SET	5.9 ± 0.6	6.6 ± 1.1	0.37
30 minutes post	3.7 ± 0.4	3.7 ± 0.3	0.75
Na^+ (mmol/L)			
15 minutes pre	136.6 ± 1.3	138.6 ± 2.5	0.24
end of SET	145.6 ± 2.5	148.2 ± 2.2	0.18
30 minutes post	138.2 ± 1.5	138.6 ± 1.5	0.75
Ca^+ (mmol/L)			
15 minutes pre	1.49 ± 0.03	1.41 ± 0.22	0.44
end of SET	1.50 ± 0.01	1.53 ± 0.03	0.21
30 minutes post	1.35 ± 0.08	1.37 ± 0.07	0.60
pCO_2 (mmHg)			
15 minutes pre	52.4 ± 2.4	53.1 ± 2.5	0.75
end of SET	63.6 ± 19.2	87.8 ± 28.6	0.23
30 minutes post	39.4 ± 3.0	39.5 ± 3.7	0.86
HCO_3^- (mmol/L)			
15 minutes pre	34.0 ± 1.8	33.2 ± 1.4	0.58
end of SET	19.5 ± 5.1	23.8 ± 3.8	0.26
30 minutes post	20.9 ± 3.9	20.9 ± 4.2	1.00

differences ($P > 0.05$) were observed between treatment groups.

Volume of Oxygen Consumption

There was no difference between the placebo and ribose treatment groups for VO_{2max} ($P > 0.05$). VO_{2max} was 165 ± 3 ml/kg/min in the placebo treatment group, and 161 ± 19 ml/kg/min in the ribose treatment group.

Heart Rate

Heart rate was not different between treatment groups at rest or during exercise ($P > 0.05$). Peak heart rate for each horse was recorded during the last exercise step, and ranged from 195 to 215 in the placebo treatment group, and from 197 to 215 in the ribose treatment group.

Experiment 3

General

The nutrient analyses of the concentrate and hay are shown in Table 17. The horses were fed 3.3 ± 0.2 kg concentrate and 2.7 ± 0.2 kg hay. The age of the eight horses are shown in Table 18. The body weight and body condition score (BCS) of the six horses are shown in Table 19.

Performance

There were no differences ($P > 0.05$) between treatment groups for total run time (Table 20).

Table 17. Nutrient analyses of the concentrate and hay[a] (Experiment 3)

Nutrient	Concentrate	Hay
Crude Protein (g/kg)	162.9	94.7
Acid detergent fiber (g/kg)	137.0	358.4
Neutral detergent fiber (g/kg)	333.2	771.1
Ether extract (g/kg)	33.4	10.1
Calcium (g/kg)	10.8	3.1
Phosphorus (g/kg)	7.3	1.9

[a] Dry matter basis

Table 18. Age of the eight horses (Experiment 3)

Horse	Age (years)
A	6
B	2
C	5
D	4
E	4
F	9
G	3
H	3

Table 19. Weight and body condition score (BCS) of horses (Experiment 3)

Horse A

Time	Weight (kg)	BCS
0 weeks	491	4.5
2 weeks	504	5.0
5 weeks	502	5.0
7 weeks	504	5.0
9 weeks	501	5.0
11 weeks	502	5.0
1st SET	497	5.0
2nd SET	493	5.0

Horse B

Time	Weight (kg)	BCS
0 weeks	469	4.0
2 weeks	488	4.0
5 weeks	486	4.5
7 weeks	487	4.5
9 weeks	487	4.5
11 weeks	488	5.0
1st SET	489	5.0
2nd SET	493	5.0

Horse C

Time	Weight (kg)	BCS
0 weeks	476	4.0
2 weeks	489	4.5
5 weeks	496	4.5
7 weeks	493	4.5
9 weeks	484	4.5
11 weeks	486	5.0
1st SET	489	5.0
2nd SET	495	5.0

Horse D

Time	Weight (kg)	BCS
0 weeks	521	4.5
2 weeks	532	4.5
5 weeks	519	4.5
7 weeks	520	4.5
9 weeks	516	4.5
11 weeks	521	4.5
1st SET	519	4.5
2nd SET	522	4.5

Table 19. Continued

Horse E

Time	Weight (kg)	BCS
0 weeks	571	5.0
2 weeks	592	5.5
5 weeks	572	5.0
7 weeks	576	4.5
9 weeks	571	4.5
11 weeks	572	5.0
1st SET	571	5.0
2nd SET	568	5.0

Horse F

Time	Weight (kg)	BCS
0 weeks	577	5.0
2 weeks	589	5.0
5 weeks	570	5.0
7 weeks	574	5.0
9 weeks	575	5.0
11 weeks	582	5.0
1st SET	578	5.0
2nd SET	585	5.0

Horse G

Time	Weight (kg)	BCS
0 weeks	460	3.5
2 weeks	467	3.5
5 weeks	480	4.0
7 weeks	482	4.0
9 weeks	498	4.5
11 weeks	513	5.0
1st SET	498	5.0
2nd SET	500	5.0

Horse H

Time	Weight (kg)	BCS
0 weeks	541	5.0
2 weeks	544	5.0
5 weeks	535	5.0
7 weeks	522	4.5
9 weeks	522	4.5
11 weeks	534	5.0
1st SET	526	5.0
2nd SET	527	5.0

Table 20. Influence of ribose supplementation on the duration of exercise during a standardized exercise test (SET) on the treadmill (Experiment 3)

Horse	Placebo		Ribose	
	Speed[a] (m/s)	Time[b] (s)	Speed[a] (m/s)	Time[b] (s)
A	11	60	12	50
B	12	30	12	60
C	13	35	12	50
D	12	15	13	05
E	12	50	13	05
F	12	25	11	60
G	12	05	12	05
H	12	40	12	20

[a] Highest speed attained

[b] Length of time at the designated speed at the end of the SET

Blood Ammonia

There were no differences ($P > 0.05$) for blood ammonia between treatment groups (Table 21). Peak blood ammonia occurred at 15 minutes post-exercise and was 334.3 ± 34.5 μmol/L in the placebo treatment group, and 322.5 ± 39.1 μmol/L in the ribose treatment group.

Plasma Lactic Acid

There were no differences ($P > 0.05$) for plasma lactic acid between treatment groups (Table 22). Peak plasma lactic acid occurred at 15 minutes post-exercise and was 26.5 ± 0.9 mmol/L in the placebo treatment group, and 26.2 ± 1.1 mmol/L in the ribose treatment group.

Plasma Glucose

There were no differences ($P > 0.05$) for plasma glucose between treatment groups (Table 23). Peak plasma glucose occurred at 15 minutes post-exercise and was 6.96 ± 0.19 mmol/L in the placebo treatment group, and 7.18 ± 0.25 mmol/L in the ribose treatment group.

Volume of Oxygen Consumption

Due to difficulties with the equipment the VO_2 of Horse C was excluded from this experiment. There was no difference between the placebo and ribose treatment groups for VO_{2max} ($P > 0.05$). VO_{2max} was 151 ± 9 ml/kg/min in the

Table 21. Influence of ribose supplementation on blood ammonia (μmol/L) in exercising horses (Experiment 3)

Time and speed	Placebo	Ribose	p-value
0 minutes pre	35.7 ± 5.0	33.6 ± 5.7	0.47
8 m/s	40.5 ± 12.2	39.9 ± 11.9	0.79
9 m/s	43.7 ± 9.7	41.5 ± 10.9	0.12
10 m/s	53.4 ± 11.7	47.6 ± 14.0	0.38
11 m/s	106.4 ± 76.3	75.6 ± 24.5	0.35
12 m/s	132.6 ± 77.5[a]	204.8 ± 84.6[b]	0.73
15 minutes post	334.3 ± 97.4	322.5 ± 110.7	0.77
30 minutes post	258.0 ± 90.1	233.1 ± 91.6	0.48

[a] Only two horses reached this speed step

[b] Only five horses reached this speed step

Table 22. Influence of ribose supplementation on plasma lactic acid (mmol/L) in exercising horses (Experiment 3)

Time and speed	Placebo	Ribose	p-value
0 minutes pre	0.7 ± 0.1	0.6 ± 0.1	0.05
8 m/s	5.0 ± 1.3	5.3 ± 1.9	0.64
9 m/s	9.0 ± 2.0	9.4 ± 2.7	0.59
10 m/s	13.2 ± 3.8	12.6 ± 3.4	0.78
11 m/s	19.4 ± 5.5	17.6 ± 3.9	0.52
12 m/s	19.8 ± 6.1[a]	23.8 ± 3.8[b]	0.69
15 minutes post	26.5 ± 2.6	26.2 ± 3.2	0.85
30 minutes post	20.7 ± 3.5	19.7 ± 3.2	0.55

[a] Only two horses reached this speed step

[b] Only five horses reached this speed step

Table 23. Influence of ribose supplementation on plasma glucose (mmol/L) in exercising horses (Experiment 3)

Time and speed	Placebo	Ribose	p-value
0 minutes pre	4.97 ± 0.35	5.01 ± 0.53	0.82
8 m/s	4.76 ± 0.24	4.84 ± 0.42	0.54
9 m/s	4.69 ± 0.21	4.84 ± 0.37	0.26
10 m/s	4.81 ± 0.31	4.95 ± 0.44	0.42
11 m/s	4.94 ± 0.33	4.99 ± 0.42	0.76
12 m/s	4.86 ± 0.35[a]	5.28 ± 0.56[b]	0.59
15 minutes post	6.96 ± 0.53	7.18 ± 0.70	0.37
30 minutes post	6.31 ± 0.56	6.69 ± 0.72	0.13

[a] Only two horses reached this speed step

[b] Only five horses reached this speed step

placebo treatment group, and 150 ± 8 ml/kg/min in the ribose treatment group.

Heart Rate

Heart rate was not different between the treatment groups at rest or during exercise ($P > 0.05$). Peak heart rate of each horse was recorded during the last exercise step, and ranged from 212 to 226 in the placebo treatment group, and from 214 to 226 in the ribose treatment group.

CHAPTER 4
DISCUSSION

Training and Performance

The current study was designed to evaluate the effects of ribose supplementation on selected metabolic parameters and performance in Thoroughbred horses performing a SET. The training protocol of 12 (Experiment 1) and 11 (Experiment 3) weeks was designed so that the horses could reach the desired fitness level of being able to gallop at 8 m/s for 3.0 km on the treadmill without becoming exhausted. This training protocol has been employed by the College of Veterinary Medicine, University of Florida, for several years, and it has been shown to be effective in conditioning Thoroughbred horses on the treadmill.

It was hypothesized that the exercise performed by the horses during the supplementation periods (training four times per week, twice on the flat and twice on 6° incline) would be sufficient in both intensity and volume to significantly reduce the available stores of muscle ATP. It was anticipated that ribose supplementation would increase the *de novo* synthesis rates of ATP, and that the increased availability of ATP would be reflected in significant difference in performance between the two treatment groups. However, the results from this study, in each of the three experiments, show that the performance between the two treatment groups was not significantly different.

In Experiment 1, one of six horses increased its run time, two of six horses had the same run time, and three of six horses decreased their run time after ribose supplementation. In Experiment 2, three of five horses increased their run time, one of five horses had the same run time, and the other horse decreased its run time after ribose supplementation. In Experiment 3, four of eight horses increased their run time, one of eight horses had the same run time, and three of eight horses decreased their run time after ribose supplementation.

The inability to detect differences between the treatment groups may be explained by the large coefficient of variation in run time between SET of the same horse. Researchers have indicated that some horses become over accustomed with the SET procedure, and may lose the anticipation of performing. Also, it was noted that some horses may learn to quit running on the treadmill even though, metabolically, they could still continue to run (Hodgson and Rose, 1994; Marlin and Nankervis, 2002).

Another plausible alternative hypothesis could be that the exercise intensity during the supplementation period may not have been sufficient to adequately deplete the ATP and total adenine nucleotide pool to a point where ribose supplementation would be proven to be beneficial.

Metabolic Measurements

In Experiment 1, blood ammonia was significantly lower in the ribose treatment group at 30 minutes post-exercise when compared to the placebo treatment group. Because it has been reported that an ammonia concentration increase correlates with the loss of ATP during exercise (Sewell and Harris, 1992; Harris et al., 1997), this may indicate that that ribose minimized the net loss of the total adenine nucleotide pool from the myocyte. Moreover, Harris et al. (1997) reported that ammonia accumulation in the blood is preferable to other catabolites (i.e., hypoxanthine, uric acid) in indicating adenine nucleotide degradation.

In Experiment 1, plasma lactic acid was numerically lower in the ribose treatment group during recovery, with the same horses having a numerically higher pH. Plasma lactic acid increases exponentially during exercise and especially when the heart rate exceeds 200 beats/minute

(Schuback and Essen-Gustavsson, 1998). The plasma lactic acid concentrations observed in these experiments resulted in the venous blood pH declining to about 7.0. It is possible that the decrease in pH contributed to muscle fatigue by inhibiting the excitation-contraction coupling (Blanchard et al., 1984; Spriet et al., 1987), reducing cross-bridge cycling (Cooke et al., 1988), and inhibiting rate-limiting enzymes controlling glycolytic flux (Trivedi and Danforth, 1966). Acidosis in muscle can lead to impairment of glycolysis and the respiratory capacity of the mitochondria, and both may be associated with a decline in muscle ATP (Roneus and Essen-Gustavsson, 1997). Evidence for a disturbance in mitochondrial function comes from both biochemical (Gollnick et al., 1990) and ultrastructural studies on equine muscle samples collected immediately after high intensity exercise and during recovery (McCutheon et al., 1992). Following exercise, a variable swelling of mitochondria with rounding and increased prominence of individual cristae occurs. Restoration to normal ultrastructural appearance occurs about 1 hour after completion of exercise and at the same time as muscle pH and temperature return to normal.

Muscle lactic acid formation occurs before ammonia accumulation (Dudley and Terjung, 1985; Katz et al., 1986;

Sewell and Harris, 1992), and it has been suggested that muscle pH has to decrease to about 6.6 in order to activate the AMP deaminase. In Experiment 1, the lower plasma lactic acid levels in the ribose treatment group may have decreased the activity of AMP deaminase, which could explain the observed lower blood ammonia levels.

The association between AMP deaminase and lactic acid can be observed from the results of the current experiments. In experiments 1 and 3, peak plasma lactic acid was ~25 mmol/L, but in Experiment 2, peak plasma lactic acid was ~20 mmol/L. This difference in plasma lactic acid concentration is reflected in the difference in the ammonia concentrations between experiments. In experiments 1 and 3, peak ammonia was above 300 µmol/L, but in Experiment 2, peak ammonia was just above 200 µmol/L.

Experiment 2 investigated the effects of a single dose of ribose (250 grams) given to horses one hour prior to the SET. No significant differences were observed between treatment groups for blood ammonia, plasma lactic acid, plasma glucose, VO_{2max}, and heart rate. There were no treatment differences for pH, K^+, Na^+, Ca^+, pCO_2, and HCO_3^- obtained at 15 min pre-exercise, at the end of the SET, and at 30 min post-exercise. It is important to note that despite the high amount of ribose given to the horses one hour prior to exercise, there were no significant increases or decreases in plasma glucose concentrations prior to, during, or after exercise.

Experiment 3 was designed similar to Experiment 1, because the results of Experiment 1 indicated that daily oral ribose supplementation may have been beneficial to exercising Thoroughbred horses. In Experiment 3, the amount of ribose given to horses was doubled so that the amount being used was comparable to the amount of ribose used in human studies. Despite the differences observed in Experiment 1 for blood ammonia and plasma lactic acid, there were no differences between treatment groups for these two metabolites in Experiment 3.

In all three experiments peak blood ammonia and plasma lactic acid concentration was detected at 15 min post-exercise. At 30 min post-exercise ammonia and lactic acid concentration decreased when compared to 15 min post-exercise, but were higher than the samples taken prior to exercise. It is possible that, had blood samples been taken for a longer time after the end of the SET, differences might have been detected.

Plasma glucose concentrations did not differ between treatment groups in any of the three experiments, indicating that ribose supplementation did not cause changes in glucose kinetics under the conditions of these experiments.

In all of the current experiments, the heart rate of each horse was increased as the speed of the treadmill was increased. The highest heart rate was recorded during the last exercise step, The heart rates observed in this study were similar to the heart rates reported by other researchers for horses performing similar SET (Hodgson and Rose, 1994; Marlin, 2002). Heart rate did not differ significantly between treatment groups at rest or during exercise in any of the three experiments.

Conclusions

Few researchers have attempted to document the effects of oral ribose supplementation as an ergogenic aid in exercising humans, but the results from these studies have been equivocal. Raue et al. (2001) reported that ribose supplementation significantly increased power output in the last of a series of sprint sessions. Also, ribose supplementation increased mean power over a five-day training period when compared to placebo (Raue et al., 2001). Van Gammeren et al. (2002) reported that ribose supplementation resulted in significant increases in muscular strength and total work performed in recreational bodybuilders. However, Op'T Eijnde et al. (2001) reported that ribose supplementation had no beneficial impact on post-exercise muscle ATP recovery or maximal intermittent exercise performance. Berardi and Ziegenfuss (2003) reported that ribose supplementation did not have a consistent or substantial effect on anaerobic cycle sprinting. Kreider et al. (2003) reported that oral ribose supplementation did not affect anaerobic exercise capacity or metabolic markers in trained humans performing anaerobic sprint tests on a cycle ergometer.

The hypotheses investigated in this study were that oral ribose supplementation could be used as an ergogenic aid during high intensity exercise. The results from this study demonstrate that ribose supplementation, in the diet of conditioned Thoroughbred horses, did not benefit the horses during a SET. In Experiment 1, blood ammonia was significantly lower in the ribose treatment group at 30 minutes post-exercise. However, in Experiment 2 and 3 no significant differences were observed for blood ammonia. There were no differences detected between the placebo and ribose treatment groups either prior to exercise, during exercise, or 30 min into recovery for either performance or any of the other metabolic markers measured. Based on these results, ribose supplementation did not show that can consistently be used as an effective ergogenic aid for exercising Thoroughbred horses.

Future Research Directions

It is possible that the exercise intensity used in this experiment was insufficient to elucidate a decrease in the ATP and the total adenine nucleotide levels. Future experiments should investigate the effectiveness of oral ribose supplementation in horses performing a more intense exercise protocol during the supplementation period. Another aim would be to further the biochemical information gathered by collecting muscle biopsies and analyzing them for ATP and total adenine nucleotide levels. This could document whether ribose supplementation induces a significant increase in these metabolites. Recently, an assay for measuring ribose in the blood has been developed. Therefore, in future studies, blood ribose concentration could be measured to examine whether oral ribose supplementation actually causes a significant increase in the circulating concentration of ribose in the bloodstream of Thoroughbred horses.

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Mr. Andreas Kavazis is a native of Cyprus, which is a Greek island in the Mediterranean Sea. Following graduation from high school he joined the Cypriot Army for two years where he was a mortar specialist. After his service in the army, he came to the United States where he graduated from Louisiana State University on May 1999 with a Bachelor of Science degree in Animal Science. After graduation he enrolled at the University of Florida, where he completed the Master of Science in Animal Science on August 2001. After receiving his degree, he continued his studies at the University of Florida to obtain a Ph.D. with a major in Animal Sciences and a minor in Exercise and Sport Sciences.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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